International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 9, Issue 3, 2017

Original Article

EVALUATION OF ANTIHYPERLIPIDEMIC, ANTI-INFLAMMATORY, AND ANALGESIC ACTIVITIES OF *EURYCOMA LONGIFOLIA* IN ANIMAL MODELS

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Received: 21 Nov 2016 Revised and Accepted: 17 Jan 2017

ABSTRACT

Objective: To investigate the anti-hyperlipidemic, anti-inflammatory and analgesic properties of of *E. longifolia* root extract in animal models.

Methods: In this study, glucose-fructose enriched diet-induced hyperlipidemia, carrageenan-induced paw edema and acetic acid-induced writhing were used to evaluate the anti-hypertriglyceridemia, anti-inflammatory and analgesic activities, respectively. At the end of the experiment of glucose-fructose enriched diet-induced hyperlipidemia, blood samples were collected and estimation of blood lipids were carried out. Edema thickness was measured using digital caliper at 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after carrageenan injection. The number of abdominal writhing for each mouse was observed and counted during a period of 1 h post injection of acetic acid.

Results: *E. longifolia* root extract demonstrated a significant reduction of triglyceride levels ($p<0.05$) compared with the control group in glucose-fructose enrich diet in rats. In anti-inflammatory test, the extract significantly inhibited the carrageenan induced paw edema formation ($p<0.05$). The extract also significantly decreased the number of writhing in acetic acid-induced mice ($p<0.05$).

Conclusion: *E. longifolia* root extract shown a significant anti-hypertriglyceridemia, anti-inflammatory and analgesic activities. Further studies are needed to determine mechanisms for its acitivities of *E. longifolia* root extract.

Keywords: *Eurycoma longifolia*, Antihypertriglyceridemia, Anti-inflammatory, Analgesic

DOI: http://dx.doi.org/10.22159/ijpps.2017v9i3.16366

INTRODUCTION

Obesity and the metabolic syndrome continue to plague the world at an alarming rate. Those will cause both substantial socio-economic and physical burden in society. WHO reported that in 2014, over 1.9 billion adults were estimated to be overweight and more than 600 million obese [1].

Recently people have been using natural products and plant to treat a wide variety of clinical disease. One of the most popular traditional plants *Eurycoma longifolia* Jack (Simaroubaceae) is a well-known folklore herbal medication in Southeast Asia. Wide spectrum of pharmacological activities of *E. longifolia* has been reported [2-5]. Despite wide range of traditional uses known, it is most solely for its aphrodisiac and anti-malarial properties [5-7]. Previous research shows that extracts from the roots of *E. longifolia* suppressed intracellular lipid accumulation in 3T3-L1 adipocytes, a treatment target for an anti-obesity agent [8]. Based on the previous study and traditional use, the present investigation was carried out to evaluate the antihyperlipidemic, anti-inflammatory and analgesic activities of *E. longifolia* root extract in animal models.

MATERIALS AND METHODS

Plant material and chemicals

E. longifolia was supplied by Merapi Farma Herbal Co. (Batch No. SL.1A.2015. PB; Yogyakarta, Indonesia) and were collected from Kalimantan Island Indonesia. The voucher specimen was deposited at the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy Sanata Dharma University in Yogyakarta Indonesia. The following chemicals were used: sodium carboxy methyl cellulose/CMC-Na (Brataco Chemika, Indonesia); glucose, fructose, methanol and acetic acid (E. Merck, Darmstadt Germany; and carrageenan (Sigma Chemical Company). Diagnostic kit for the estimation of Cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglyceride (TG) kits were purchased were from Roche Diagnostics GmbH, Mannheim, Germany. All other chemical were of analytical grade and were purchased from E. Merck, Darmstadt, Germany. Instruments used in this study included Cobas C501 (Roche Diagnostics) for serum chemistry analysis.

Preparation of *E. longifolia* root extract

The root of *E. longifolia* were powdered and extracted with 95% (v/v) methanol for 48 h at room temperature. The methanol extract was then distilled, evaporated to obtain semisolid *E. longifolia* root extract (EL) (yield 9.32%) and re-dissolved in CMC-Na (1% w/v).

Test animals and housing

28 adult male Wistar rats (150-250 g), and 25 adult male and 25 female Swiss mice (20-30 g) were obtained from the Imono Laboratory, Sanata Dharma University, Indonesia. The animals were maintained under standard laboratory condition. They were housed in standard cages (five animals per cage) at temperature 22 °±2 °C and 12:12h light dark cycle. The animals were provided with pelleted diet as normal diet or glucose-fructose enriched diet (GFED) and water ad libitium. All procedures described were reviewed and approved with approval number KE/KF/337/EC by Medical and Health Research Ethics Committee Faculty of Medicine Gadjah Mada University Yogyakarta Indonesia.

Antihyperlipidemic study

Healthy male rats were fasted overnight and randomly divided into four groups each containing 7 animals. The control group was fed normal diet until the end of treatment. The remaining groups were fed GFED for 42 d [9]. Following confirmation of GFED-induced hypertriglyceridemia, the three groups were then divided into group I (EL 75 mg/kg b/w), group II (EL 150 mg/kg b/w) [10], and group GFED-control (continued with vehicle). All treatments were continued for 5 d following oral administration. At the end of treatment, rats in all groups were anaesthetized with ketamine. The blood was collected from all groups of rats by retro-orbital sampling for serum chemistry analysis. Serum lipid profile tests were measured using commercial kits (Roche Diagnostics).

Anti-inflammatory study

Male Swiss mice were divided into five groups randomly (negative control, positive control and dose groups), consisting of 5 mice in each group. Acute edema was induced by the injection of carrageenan1% (prepared in normal saline) into the sub-plantar region of hind-paw of mice [11]. Then, each group was treated orally with 1% CMC-Na (negative control), 4.48 mg/kg BW of diclofenac sodium (positive control), and treatment doses of 105, 210 and 420 mg/kg BW EL [10]. Edema thickness was measured using digital caliper at 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after carrageenan injection [12, 13]. The calculation of the edema volume was conducted using formula area under curve (AUC) and percentage of inhibition of inflammation [13-15].

Area under curve (AUC) was calculated for each minute within 0-6 h using trapezoid method formula, as below:

$$AUC_{tn-1}^{tn} = \frac{T_{tn-1} + T_{tn}}{2} (t_n - t_{n-1}) \quad [16]$$

T_{tn-1}: Average edema volume on t_{n-1}

T_{tn}: Average edema volume on t_n

Analgesic study

The analgesic activity of EL was tested using acetic acid-induced writhing method [11, 14, 15, 17]. Female mice Swiss were divided randomly into five groups (n=5). Group I as negative control received 1% CMC-Na, group II as positive control received 91 mg/kg BW of aspirin, group III-V received 105, 210 and 420 mg/kg BW of EL respectively. After 5 min of treatment, all animals were administered 1% with 50 mg/kg BW of acetic acid intraperitoneally. The total number of writhes was recorded at intervals of 5 min for a period of 1 h. The calculation of percentage of inhibition using the following ratio: (control mean–treated mean)/control mean x 100 [14, 15].

Statistical analysis

Results are expressed as mean±standard deviation (SD). Data were analyzed using one-way analysis of variance followed by *post-hoc* Tukey HSD tests using SPSS 22. A p-value<0.05 was considered statistically significant. Statistical differences were determined using the Student's t-test, with p-values being indicated for each fig.

RESULTS

Effect of *E. longifolia* root extract in hyperlipidemia-induced rats

The administration of GFED significantly increased (p<0.05) TG levels up to 186.1% and decreased high-density lipoprotein (HDL) levels by 43.0% (table 1). GFED did not increase serum total cholesterol or low-density lipoprotein (LDL) levels.

Daily administration of both doses of EL for 5 d led to a significant reduction (p<0.05) in TG compared with the GFED group. There was no significant difference in HDL levels among all doses of EL and those of the GFED group.

Table 1: Effect of *E. longifolia* root extract on lipid parameters in rats feeds with glucose-fructose enrich diet (GFED)

Treatment	Cholesterol (mmol/l)	Triglyceride (mmol/l)	HDL-c (mmol/l)	LDL-c (mmol/l)
Normal diet	1.74±0.16	1.08±0.09[b]	1.65±0.08[b]	0.21±0.05
GFED	1.86±0.15	3.08±0.21[a]	0.94±0.07[a]	0.24±0.04
GFED+EL 75 mg/kgBW	1.69±0.26	2.69±0.34 [a,b]	0.92±0.07[a]	0.25±0.06
GFED+EL 150 mg/kgBW	1.88±0.17	0.43±0.05[a,b]	0.70±0.08[a,b]	0.37±0.07 [a,b]

Values are expressed as mean±SD of seven animals in each group; a: p<0.05 vs normal diet; b: p<0.05 vs GFED

Effect of *E. longifolia* root extract on carrageenan-induced mice

The anti-inflammatory activity of *E. longifolia* root extract against carrageenan induced paw edema showed that the extracts exhibit significantly (p<0.05) and dose-dependently reduced the paw edema swelling (table 2). The percentage inhibition in the paw edema in mice treated with *E. longifolia* root extract was found to be 15.7; 22.0 and 26.8% at the dose of 105; 210 and 420 mg/kg BW respectively.

Table 2: Effect of *E. longifolia* root extract on carrageenan-induced mice

Treatment	AUC	% inhibition
Negative control (CMC Na)	426.7 ± 18.3[b]	0.0
Positive control (diclofenac sodium 4.48 mg/kg BW)	255.9 ± 16.8[a]	40.0
EL 105 mg/kg BW	359.6 ± 5.8[a,b]	15.7
EL 210 mg/kg BW	333.0 ± 5.6[a,b]	22.0
EL 420 mg/kg BW	312.4 ± 4.0[a,b]	26.8

Values are expressed as mean±SD of five animals in each group; a: p<0.05 vs negative control; b: p<0.05 vs positive control

Effect of *E. longifolia* root extract on acetic acid-induced writhing in mice

Table 3 showed the effect of *E. longifolia* root extract on acetic acid-induced writhing in mice. All of the doses of *E. longifolia* root extract produced significant (p<0.05) reduction of writhing by the acetic acid in a dose dependent manner.

The percent inhibition of 105, 210 and 420 mg/kg BW was 25.4; 57.3 and 59.0% for *E. longifolia* root extract respectively.

Table 3: Effect of *E. longifolia* root extract on acetic acid induced writhing in mice

Treatment	Number of writhing	% inhibition
Negative control (CMC Na)	57.6 ± 1.1[b]	0.0
Positive control (aspirin 91 mg/kgBW)	16.2 ± 1.3[a]	71.9
EL 105 mg/kg BW	43.0 ± 1.0[a,b]	25.4
EL 210 mg/kg BW	24.6 ± 2.1[a,b]	57.3
EL 420 mg/kg BW	23.6 ± 4.2[a,b]	59.0

Values are expressed as mean±SD of five animals in each group; a: p<0.05 vs negative control; b: p<0.05 vs positive control

DISCUSSION

Several studies have reported that a high carbohydrate diet is responsible for the development of hypertriglyceridemia in rodent animal models [9, 18-20]. Hypertriglyceridemia occurs because high fructose in the blood leads to increased *de novo* hepatic fatty acid synthesis and, subsequently, this releases a high amount of TG [21]. The *E. longifolia* root extract used in our study reduced TG levels induced by glucose-fructose enrich diet. Therefore, the *E. longifolia* root extract has a potent anti-hypertriglyceridemia activity in rats. These reductions of TG may be associated with a previous *in vitro* report in which *E. longifolia* suppressed lipid accumulation in 3T3-L1 adipocytes [8].

Carrageenan, as irritant substances, induced inflammation in biphasic event. The initial phase is associated to the release of serotonin, histamine, and bradykinin; while the late phase is attibuted to the release of prostaglandin and induce of cyclooxygenase that increasing vascular permeability and the neutrophil infiltration into the inflammatory site and production of free radicals that cause edema [22]. In our results, the *E. longifolia* root extract significantly inhibited paw edema-induced carrageenan in all the dose level.

Additionally, the *E. longifolia* root extract showed significant analgesic action at all dose levels (105, 201 and 420 mg/kg BW). Analgesic effect was evaluated using acetic acid-induced writhing test in mice. Acetic acid injection has been associated with increased level of E and F prostaglandins in peritoneal fluids as well as lipoxygenase products [23]. The significant reduction of *E. longifolia* root extract might be due to the presence of analgesic principles acting with the prostaglandin pathways.

It has been reported that *E. longifolia* contains quassinoids, triterpene, biphenylneolignan and alkaloid [7, 24-26]. The presence of eurycomaloactone, 14,15β-dihydroklaieanone and 13, 21-dehydroeurycomanone in *E. longifolia* root were identified as potent NF-κB inhibitors [27, 28]. They act by inactivation of the NF-κB signaling pathway, a pro-inflammatory transcriptional factor. Therefore, their inhibition results in anti-inflammatory effect. Anti-inflammatory and analgesic effects of *E. longifolia* root extract in this study are in agreement with a previous study. Han *et al.* demonstrated that methanolic extract of *E. longifolia* root has a potential analgesic agent on both heat-induced pain and chemical induced pain in hot plate test and acetic acid-induced writhing test, repectively. Methanolic extract of *E. longifolia* root also has anti-inflammatory agent on carrageenan-induced paw edema [10].

CONCLUSION

In conclusion, we can confirm that *E. longifolia* root extract are endowed with anti-hypertriglyceridemic, anti-inflammatory and analgesic properties that support to the traditional use of this plant. However, further study is needed to investigate the mechanisms of pharmacological effects.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest

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How to cite this article

- Phebe Hendra, Fenty, Putu Ririn Andreani, Bernadetha Maria Estika Pangestuti, Jeffry Julianus. Evaluation of anti-hyperlipidemic, anti-inflammatory, and analgesic activities of *Eurycoma longifolia* in animal models. Int J Pharm Pharm Sci 2017;9(3):166-169.